

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the patent application of David Hone

Serial No. 10/632,095

Group Art Unit 1632

Filed 08/01/2003

Examiner: Licto

For: ***"DNA VACCINES THAT EXPRESS MUTANT ADP-RIBOSYLTRANSFERASE TOXINS WHICH DISPLAY REDUCED, OR ARE DEVOID OR, ADP-RIBOSYLTRANSFERASE ACTIVITY"***

Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

1. I am the inventor of the above-identified application, and I am qualified to design and conduct experiments related to DNA vaccines, particularly as they relate to the claimed invention. As evidence of my qualifications, attached hereto is a copy of my resume.

2. I am an employee of Aeras, the assignee of record for the above-identified application.

3. I have reviewed the subject patent application, including the claims, and the Examiner's remarks as contained in the Office Action mailed on November 3, 2004.

4. Attached here as Exhibit A is a description (4 pages of text and 1 figure) of experiments to test the ability of a composition of genes encoding an L41F mutant cholera toxin and an antigen to elicit an immune response in a mammal, as is contemplated by the claimed invention. The experiments were carried out under my direct supervision. The results showed that compositions containing DNA encoding cholera toxin L41F mutant CtxA1_{S41Y} display

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substantive adjuvant activity and augment both the magnitude and persistence of the serum IgG response to the HIV antigen gp120, in comparison to the control composition. Further, no adverse (toxic) affects were observed as a result of the administration of the composition.

5. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-referenced application and any patent issuing thereon.

Date 2-3-5

Signed



David Hone



Exhibit A

Example

Evaluation of the adjuvant properties of

S63Y and S41Y derivatives of CtxA1

Experimental aim

The aim of this experiment was to evaluate the adjuvant activity of DNA vaccines that encode S63Y and S41Y derivatives of CtxA1 in plasmids designated pRc-CtxA1_{S63Y} or pRc-CtxA1_{S41Y}.

Methods

Construction of DNA vaccines

Plasmid pRc/CMV (Genebank accession E14286) was purchased from InVitrogen Corporation (San Diego, CA). DNA vaccine pOGL1 is described elsewhere (4).

DNA vaccines encoding S63Y and S41Y derivatives of CtxA1, designated pRc-CtxA1_{S63Y} or pRc-CtxA1_{S41Y}, were constructed using the QuikChange[®] Site-Directed Mutagenesis Kit (Catalog #200518, Stratagene) according to the manufacturer's directions. The site-directed mutagenesis process entailed whole-plasmid PCR using CtxA1 DNA vaccine, pRc/CMV-CTA (2), as template. The resultant PCR-generated plasmid was digested with *DpnI* to remove the template DNA and the digested DNA was introduced into *E. coli* Stable2[®] by chemical transformation. The transformed bacilli were cultured on tryptic soy agar (Difco, Detroit MI) supplemented with 100-μg/ml ampicillin at 30°C for 16 hr.

Isolated colonies were selected and grown overnight in 3 ml of LB medium supplemented with 100 μg/ml ampicillin. DNA was extracted from overnight liquid

Exhibit A

cultures using a Qiagen mini plasmid DNA preparation kit (Cat No Q7106). Plasmid PCR using primers specific for ctxA1_{S63Y} or ctxA1_{S41Y}, and agarose gel electrophoresis were conducted to screen for an appropriate derivative; clones that tested positive for were stored at -80°C and used as the source DNA for the vaccination studies below.

Animal study methods

Specific-pathogen free, 6-8 week old female BALB/cJ mice, purchased from The Jackson Laboratory (Bar Harbor, ME), were maintained in a specific-pathogen free, micro-isolator environment, and allowed to drink and eat *ad lib*. Groups of 5 mice were vaccinated intramuscularly with admixtures containing 30 µg each of endotoxin-free (<0.5 EU per mg of DNA) pOGL1 + pRc/CMV, pOGL1 + pRc-CtxA1_{S63Y} or pRc-CtxA1_{S41Y} DNA suspended in normal saline (0.85% (w/v) NaCl), as described (5).

Humoral responses to gp120

Solid-phase ELISA was used to determine gp120-specific Ab titers in the sera. Briefly, 96 well microtiter plates (Nunc, Rochester, NY) were coated with 100 µl of 3 µg/ml of HIV-1_{MN} gp120 (ViroStat, Portland, Oregon) in PBS at 4°C overnight. Plates were washed three times with TBS then blocked with blotto (5% wt/vol nonfat dried milk in TBS) at room temperature for 1 hour. Serially diluted sera were added to the wells and incubated at room temperature for 2 hours then washed three times with TBS. Alkaline phosphatase-conjugated goat anti-mouse IgG (Accurate Chemical, Newington NH cat# SBA103004) diluted 1/2000 in blotto + 5% lamb serum was added and incubated at room temperature for 1 hour. The plates were washed three times with TBS before adding substrate (Gibco BRL, Carlsbad CA cat# 19589-019) and incubating for 17 minutes.

Exhibit A

Amplifier was then added (Gibco BRL cat# 19589-019) and incubated for an additional 15 minutes. Absorbance was read at 495 nm using a Victor 1420 Multilabel Counter (EG&G Wallac, Turku, Finland).

End-point titers were calculated by plotting serum dilution *VS* absorbance and taking the value where the best-fit line intersected than the mean of the negative control row plus three standard deviations.

Results

To assess the adjuvant activity of the S63Y and S41Y derivatives of CtxA1 plasmids pRc-CtxA1_{S63Y} or pRc-CtxA1_{S41Y} were constructed by site-directed mutagenesis, as described above. These constructs were determined to be enzymatically inactive by assaying transfected cells for intracellular cAMP levels by ELISA (data not shown). For the immunization studies, groups of 5 mice were immunized intramuscularly on weeks 0, 2, and 16 with admixtures containing 30 µg each of endotoxin-free (<0.5 EU per mg of DNA) pOGL1 + pRc/CMV, pOGL1 + pRc-CtxA1_{S63Y} or pRc-CtxA1_{S41Y} DNA suspended in normal saline (0.85% (w/v) NaCl), as described (5). The mice were bled monthly over an 11-month period post-priming. Sera collected before and after vaccination were used to measure the serum IgG responses to gp120 by ELISA (1, 3).

As shown in figure 1, the serum ELISAs demonstrated that the DNA vaccines encoding CtxA1_{S63Y} or CtxA1_{S41Y} both display substantive adjuvant activity and augment both the titer and duration of the serum IgG response to gp120. The gp120_{MN} DNA vaccine, pOGL1 admixed with the control vector pRc/CMV elicited serum IgG to gp120 that increased to a maximum titer of approximately 1:3,00 by 3 months after the initial

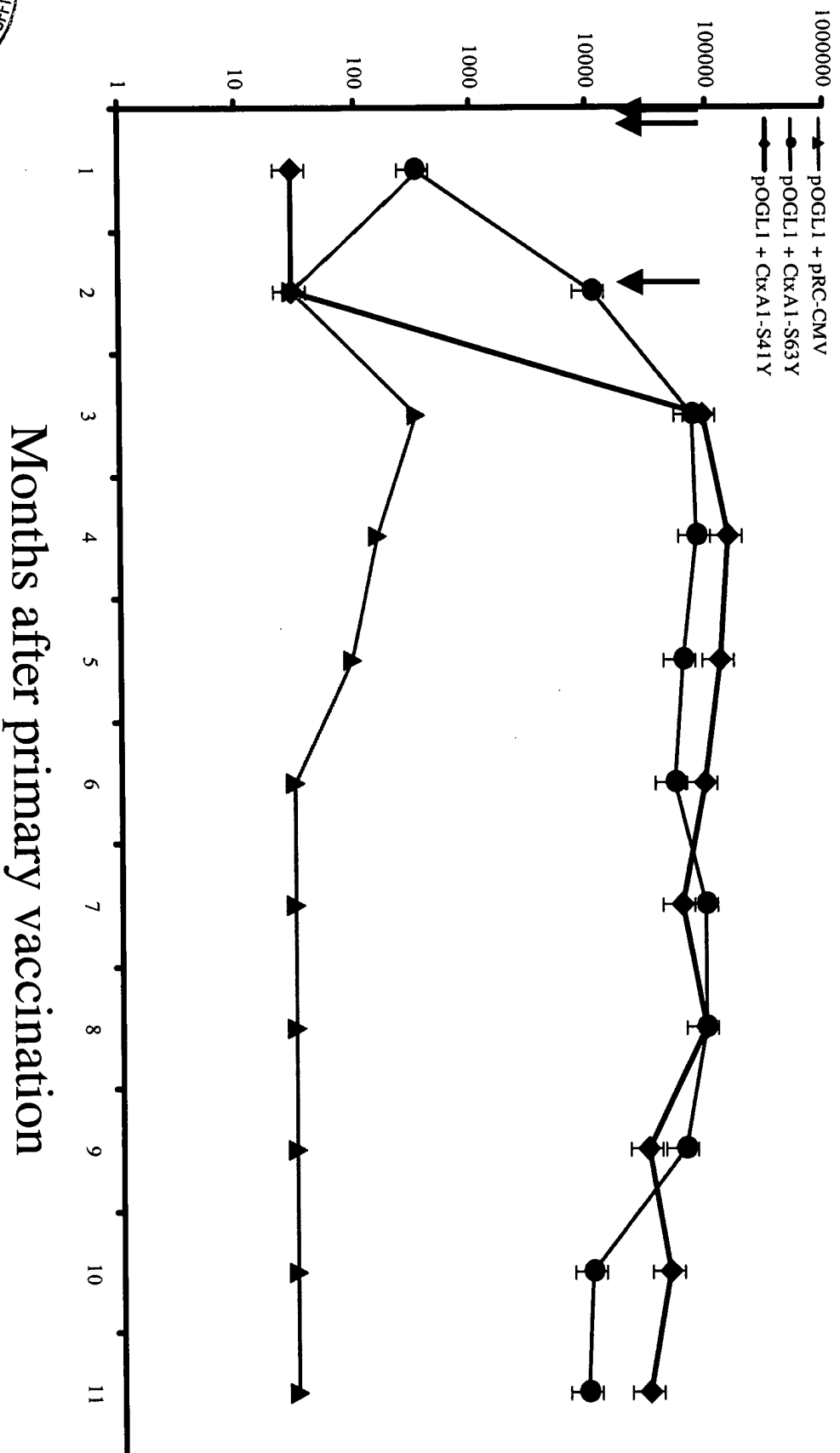
Exhibit A

vaccination and waned thereafter. By contrast, immunization with either of the two formulations that included the DNA vaccines encoding the mutant CtxA1 dramatically enhanced both the magnitude and persistence of the anti-gp120_{MN} antibody response. Thus, we conclude that DNA vaccines encoding CtxA1_{S63Y} and CtxA1_{S41Y} are capable of imparting potent adjuvant activity. It should be noted that at no time after the immunizations were any adverse affects observed at the immunization sites. This indicates that the mutant adjuvants were well tolerated; however, histologic analysis will be necessary to confirm this conclusion.

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2. Bagley, K. C., M. T. Shata, D. Y. Onyabe, A. L. DeVico, T. R. Fouts, G. K. Lewis, and D. M. Hone. 2003. Immunogenicity of DNA vaccines that direct the coincident expression of the 120 kDa glycoprotein of human immunodeficiency virus and the catalytic domain of cholera toxin. *Vaccine* 21:3335-41.
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5. Wolff, J. A., R. W. Malone, P. Williams, W. Chong, G. Acsadi, A. Jani, and P. L. Felgner. 1990. Direct gene transfer into mouse muscle in vivo. *Science* 247:1465-1468.

Exhibit A Adjuvant properties of S63 Y and S41 Y derivatives of CtxA1





BIOGRAPHICAL SKETCH

NAME:- David M. Hone

POSITION TITLE:- Chief Scientific Officer

Education and Training

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
University of Adelaide, Australia	B.Sc.(Hons.)	1984	Genetics of lipopolysaccharide biosynthesis
University of Adelaide, Australia	Ph.D.	1984-1988	<i>Salmonella</i> vaccines and vaccine vectors
University of Maryland, Baltimore, USA	Postdoc	1988-1990	<i>Salmonella</i> and <i>Shigella</i> vectors

Professional Experience

1990-1995	Assistant Professor, Division of Geographic Medicine, Department of Medicine, University of Maryland at Baltimore.
1992-1995	Chief, Salmonella Vaccine Vector Unit, Center for Vaccine Development, University of Maryland at Baltimore.
1996	Assistant Professor, Institute of Human Virology, Medical Biotechnology Center, University of Maryland Biotechnology Institute, Baltimore.
1997-2003	Associate Professor, Institute of Human Virology, UMBI, Baltimore.
1999-2003	Acting Director, Division of Vaccine Research, Institute of Human Virology, UMBI, Baltimore.
2003-Present	Chief Scientific Officer, Aeras Global TB Vaccine Foundation, Rockville, MD

Editorial Assignments

1995-1997	Editorial Board, Infection and Immunity.
1990-present	Vaccine, Proceedings of the National Academy of Sciences USA, Journal of Infectious Diseases, New England Journal of Medicine, Journal of Cellular and Molecular Biology.

Grant Review Committees

1993	Technical Review Committee, NIH-NIAID-DAIDS RFP 94-05. Collaborative Mucosal Immunology Groups for AIDS Vaccines.
1993-1996	Bacterial and Mycology Small Business Innovative Research Study Section, National Institute of Allergy and Infectious Disease.
1994-1999	Reviewer, Steering Committee on Diarrhoeal Disease Vaccines, WHO Global Program for Vaccines and Immunization. WHO, Geneva, Switzerland.
1999	Technical Review Committee, NIAID-DAIDS RFP 00-010. HIV Vaccine Design and Development Teams.
2001	Technical Review Committee, NIAID-DAIDS RFP 02-06. HIV Vaccine Design and Development Teams.
2005	Technical Review Committee, NIAID-ZRG1 VACC (01), HIV Vaccine Study Section.

Selected Publications (from a total of 62 publications and patents)

1. Onyabe D.Y., Fouts T.R., and D.M. Hone. Characterization of humoral immune responses to recombinant HIV-1 gp120 genetically linked to human CD4 and a CD4 mimetic (*Submitted*).
2. Wang X., Kochetkova I., Haddad A., Hoyt T., Hone D.M., and D.W. Pascual. 2005. Transgene vaccination using *Ulex europaeus* agglutinin I for targeted mucosal immunization against HIV-1 envelope. (*Submitted*)
3. Ahearn A.J., Kalyanaraman V.S., Popovic M., and D.M. Hone. 2005. Tat-stimulated dendritic cell supernatants promote CD8⁺ T cell activation but not cytolytic effector functions. (*Submitted*)
4. Sadoff J.C. and D.M. Hone. 2005. The role of Go No-Go decisions in TB vaccine development. *In: Microbes and Infection: Focus on rational vaccine development against tuberculosis*. S.E. Kauffman (Ed.). (*Submitted*)
5. Wang X., Hone D.M., Haddad A., Shata M.T., and D.W. Pascual. 2003. M Cell Vaccination for Pulmonary CTL. *J. Immunol.* 171:4717-4725.

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7. Bagley K.C., Shata M.T., Onyabe D.Y., DeVico A.L., Fouts T.R., Lewis G.K., and D.M. Hone. 2003. Immunogenicity of DNA vaccines that direct the coincident expression of the 120 kDa glycoprotein of HIV-1 gp120 and the catalytic domain of cholera toxin. *Vaccine*, **21**:3335-3341.
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10. Shata M.T., Lewis G.K., and D.M. Hone. 2001. Intragastric vaccination with a *Salmonella* Env DNA vaccine vector elicits mucosal HIV-1 envelope-specific CD8⁺ T cells. *Vaccine*, **20**:623-629.
11. Shata M.T. and D.M. Hone. 2001. Vaccination of mice with a *Shigella*-gp120 DNA vaccine vector induces of HIV-1 gp120-specific CD8⁺ T cells and anti-viral immunity. *J. Virol.*, **75**:9665-9670.
12. Shata M.T., Stevceva L., Lewis G.K., and D.M. Hone. 2000. Recent advances with recombinant bacterial vaccine vectors. *Molec. Med. Today*, **6**: 66-70.
13. Fouts T.R., Tuskan R., Godfrey K., Reitz M., Hone D.M., Lewis G.K., and A.L. DeVico. 2000. Expression and characterization of a single-chain polypeptide analogue of the human immunodeficiency virus type 1 gp120-CD4 receptor complex. *J. Virol.*, **74**:11427-11436.
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